inhibitors, the 4-anilinoquinazolines/4-anilinoquinolines, to introduce vibrational probes of electric field, which measure electrostatics via the vibrational Stark effect, into the ATP-binding sites of different kinases. The results yield direct insight into how chemical changes within this inhibitor class influence the selectivity for particular kinases, potentially yielding a method for rationally designing selective inhibitors.

2088-Plat
Specificity, Structure and Dynamics of Tiam1 PDZ Domain Ligand-Bound Complexes
Ernesto J. Fuentes, Xu Liu, Tyson R. Shepherd, David C. Speckhard, Ann M. Murray.
University of Iowa, Iowa City, IA, USA.
PSD-95/DlgA/ZO-1 (PDZ) domains are among the most abundant protein-protein interaction domains in the human proteome and typically bind the 4-10 C-terminal residues of its interaction partner with exquisite specificity. We used two homologous PDZ domains from the Tiam-family of guanine nucleotide exchange factors to investigate PDZ specificity. The Tiam1 and Tiam2 PDZ domains have overlapping but distinct ligand binding specificity, and this is exemplified by their unique preferences for C-terminal peptides derived from the syndecan1, Caspr4 and neurexin1 adhesion proteins. The Tiam1 PDZ domain binds syndecan1 and Caspr4 but not neurexin1, while the Tiam2 PDZ domain binds Caspr4 and neurexin1 but not syndecan1. Amino acid sequence comparison of Tiam-family PDZ domains revealed that four residues critical for ligand specificity are not conserved. Remarkably, substitution of these four residues in the Tiam1 PDZ for those found in the Tiam2 PDZ domain switched ligand specificity. To understand the structural and dynamic basis for this change in specificity we used X-ray crystallography and solution NMR methods, respectively. We determined the crystal structures of wild type Tiam1 PDZ domain bound to syndecan1 and phosphorylated syndecan1 peptides and the Tiam1 PDZ quadruple mutant (QM) bound to Caspr4 and neurexin1 peptides. Comparison of the crystal structures of the Tiam1 PDZ-syndecan1 and PDZ-phosphorylated syndecan1 showed that a distinct specificity pocket is used to accommodate the phosphoryl group. The crystal structure of the Tiam1 QM PDZ domain showed a unique side chain stacking interaction between aromatic residues in the PDZ domain and the Caspr4 ligand. Side chain methyl relaxation experiments revealed distinct patterns of dynamics in the Tiam1 PDZ-syndecan1 and PDZ-Caspr4 complexes. Collectively, the structures and dynamics of physiologically-based PDZ domain complexes are contributing to understanding the origin of PDZ specificity and function of Tiam-family PDZ domains.

Platform: Channel Regulation & Modulation

2089-Plat
Structural Basis For Alcohol Modulation of Pentameric Ligand-Gated Ion Channels
Rebecca J. Howard[1], Samuel Murail[2], Kathryn E. Ondricek[1], Pierre-Jean Corringer[3], Erik Lindahl[2], James R. Trudell[4], R. Adron Harris[1].
[1]The University of Texas at Austin, Austin, TX, USA, [2]Kungliga Tekniska Hogskolan, Stockholm, Sweden, [3]Institut Pasteur, Paris, France, [4]Stanford University School of Medicine, Stanford, CA, USA.
Despite its long history of use and abuse in human culture, the molecular basis for alcohol action in the brain is poorly understood. The recent determination of the atomic-scale structure of GLIC, a prokaryotic member of the pentameric ligand-gated ion channel (pLGIC) family, provides a unique opportunity to characterize the structural basis for modulation of these channels, many of which are alcohol targets in brain. We observed bimodal modulation of GLIC by n-alcohols, similar to some eukaryotic pLGICs: methanol and ethanol weakly potentiated proton-activated currents in GLIC, whereas n-alcohols larger than ethanol inhibited them. Mapping of residues important to alcohol modulation of ionotropic receptors for glycine, GABA, and acetylcholine onto GLIC revealed their proximity to transmembrane cavities that may accommodate one or more alcohol molecules. Site-directed mutations in the pore-lining M2 helix allowed the identification of four residues that influence alcohol potentiation, with the direction of their effects reflecting helical structure. At one of the potentiation-enhancing residues, decreased side chain volume converted GLIC into a highly ethanol-sensitive channel, comparable to its eukaryotic relatives. Covalent labeling of M2 positions with a methanethiosulfonate reagent further implicated residues at the extracellular end of the helix in alcohol binding. Molecular dynamics simulations elucidated the structural consequences of a potentiation-enhancing mutation and suggested a structural mechanism for alcohol potentiation via interaction with a transmembrane cavity previously termed the "linking tunnel." These results provide a unique structural model for independent potentiating and inhibitory interactions of n-alcohols with a pLGIC family member.

2090-Plat
Ceramide Channel Regulation by Bcl-2 Family Proteins: Molecular Insights
Meenu N. Perera, Shang-Hsuan Lin, Kaiti Chang, **Marco Colombini**.
University of Maryland, College Park, MD, USA.
The sphingolipid, ceramide can self-assemble in phospholipid membranes to form large channels capable of translocating proteins through membranes. Electronmicroscopic visualization and electrophysiological measurements reveal a range of pore sizes with the most frequent being 10 nm in diameter. The channel size and the propensity to form channels are controlled by the apoptosis-related Bcl-2 family proteins. Bcl-xL favors channel disassembly whereas Bax favors channel growth. By using ceramide analogs we have identified regions of the ceramide molecule that are recognized by these proteins and through which the proteins exert their influence. The ability of Bax to enhance ceramide channel formation was sensitive to the stereochemistry of the ceramide head group and the hydrogen bonding ability of the amide nitrogen whereas the ability of Bcl-xL to favor channel disassembly was highly sensitive to the length of the fatty acyl chain length of ceramide. It is likely that the N-acyl chain is binding to the hydrophobic pocket on Bcl-xL because the action of Bcl-xL is blocked by 2-methoxyantimycin A3, ABT-737 and ABT-263, inhibitors that specifically bind at this site. The results are consistent with the conclusion that the highly specific binding of these proteins to the ceramide channel results in structural changes that propagate throughout the channel in an allosteric manner resulting in a disturbance of the dynamic equilibrium between ceramides in the channel and ceramides in other forms in the membrane. Depending on the influence on the dynamic equilibrium channel growth or disassembly may be favored. (Supported by NSF grant: MCB-1023008)

2091-Plat
Activation of M-Type Potassium Channels by Different Membrane Phospholipids and Analogs
Vsevolod Telezhkin, Alastair J. Gibb, David A. Brown.
University College London, London, United Kingdom.
M-type (Kv7.2/7.3) channels are activated by the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PI(4,5)P_2) [Li et al., 2005: *J.Neurosci*., 25,9825], through interaction with a cluster of basic residues in the C-terminus [Hernandez et al, 2008: *J.Gen.Physiol*., 132,361]. However, little is known of the phospholipid specificity and requirements for this activation. We have explored this using inside-out membrane patches from CHO cells stably-expressing Kv7.2 and Kv7.3 subunits and held at a constant voltage *ca.* −20 mV. The dioctanoyl mono-, di- and tri-phosphatidylinositides DiC8-PI(4)P, DiC8-PI(4,5)P_2 and DiC8-PI(3,4,5)P_3 all produced biphasic P_{open}-concentration curves, maximizing at P_{open} ~0.8. EC_{50}s for the 'high-affinity' component 1 (maximum P_{open} ~0.2) were similar at ~1 μM; 'low-affinity' EC_{50}s were inversely proportional to the number of phosphates (DiC8-PI(4)P ~100 μM, DiC8-PI(4,5)P_2 ~50 μM, DiC8-PI(3,4,5)P_3 ~35 μM). In contrast, the inositol phosphates I(1,4,5)P_3 and I(4,5)P_2 neither activated nor inhibited the channels up to 300 μM, suggesting a crucial role for the lipophilic moiety. This was tested further using sphingosine-1-phosphate (S-1-P), fingolimod phosphate (FTY720-P) and 1-oleoyl lysophosphatidic acid (LPA). All three activated the Kv7.2/7.3 channels, to P_{open} values at 100 μM of ~0.8 (LPA), 0.15 (S-1-P) and 0.022 (FTY720-P). In each case 'high' and 'low' affinity components to the activation curves could be discerned, with EC_{50}s of 1.5 and 40 μM (LPA), 3 and 160 μM (S-1-P) and 0.5 and 61 μM (FTY720-P). No channel activation was observed using membrane lipids devoid of phosphate groups (D-erythrosphingosine, fingolimod, phosphatidylglycerol and phosphatidylcholine, all at 100 μM). Thus, M-channels can be activated in a rather similar manner by a range of membrane lipids, the minimal requirements at concentrations tested being one or more terminal phosphates and a lipophilic domain. Supported by Wellcome Trust grant 085419.

2092-Plat
BK Channel Modulation by Leucine-Rich Repeat Containing Proteins
Jiusheng Yan, Richard W. Aldrich.
University of Texas at Austin, Austin, TX, USA.
Molecular diversity of ion channel structure and function underlies variability in electrical signaling in nerve, muscle, and non-excitable cells. Regulation by variable auxiliary subunits is a major mechanism to generate tissue- or cell-specific diversity of ion channel function.Mammalian large-conductance, voltage and calcium-activated potassium (BK, K_{Ca}1.1) channels are ubiquitously expressed with diverse functions in different tissues or cell types, consisting of the pore-forming, voltage- and Ca^{2+}-sensing α-subunits (BKα), or together

with the tissue-specific auxiliary subunits, previously known as four β-subunits (β1 - β4). We previously identified a leucine-rich repeat (LRR) containing protein, LRRC26, as a new type of BK channel auxiliary subunit, which causes an unprecedented large negative shift in voltage dependence of channel activation. Here we report a group of LRRC26 paralogous proteins LRRC52, LRRC55 and LRRC38 that potentially function as LRRC26-type auxiliary subunits of BK channels. LRRC52, LRRC55 and LRRC38 produce marked shifts in the BK channel's voltage dependence of activation in hyperpolarization direction to different extents. They together with LRRC26 show distinct expression in different human tissues and may have a broad influence of the BK channel function in different tissues or cell types.

2093-Plat

A Modular Heat-Sensing Domain Activates K_{2P}2.1 through a Heat-Insensitive Gate

Sviatoslav Bagriantsev, Kimberly Clark, Daniel L. Minor.
University of California, San Francisco, San Francisco, CA, USA.

Ion channels often have modular structure with dedicated gating and sensory domains. Ligand binding to a localized site in the sensory domain usually affects channel function through allosteric effects on the gate. It is not clear whether this paradigm is applicable to ion channels regulated by temperature. Indeed, temperature may have an effect on the whole channel structure and thus, bypass the need for a dedicated heat-sensing domain. Heat leads to activation of the 'leak' two-pore (K_{2P}) potassium channel K_{2P}2.1 (KCNK2/TREK-1) through opening of a selectivity filter-based outer gate in the extracellular pore domain. We sought to investigate whether the pore plays a role in sensing heat or whether it only functions as a gate that responds to the commands from the temperature-sensing elements located elsewhere in the channel. We found that the incorporation of mutations designed to prevent allosteric communication between the intracellular C-terminal domain and the pore domain of K_{2P}2.1 resulted in channels that remain functional but fail to respond to temperature. These results indicate that the gating mechanism of the pore lacks intrinsic temperature sensitivity and that the heat-sensing elements of K_{2P}2.1 are confined within its C-terminus. Our data provide experimental support for the general notion of the existence of modular temperature sensing domains and highlight functional distinction between gating and sensory elements in heat-sensitive ion channels.

2094-Plat

K2P1 Assembles with K2P3 or K2P9 to Form SUMO-Regulated Task Background Channels in Cerebellar Granule Neurons

Leigh D. Plant[1], Leandro Zuniga[2], Dan Araki[2], Jeremy D. Marks[2], Steve A.N. Goldstein[1].
[1]Brandeis University, Waltham, MA, USA, [2]University of Chicago, Chicago, IL, USA.

Cell-surface K2P1 channels are most often electrically quiet despite their widespread expression in excitable tissues. Previously, we studied human K2P1 expressed in Chinese hamster ovary cells and found one small ubiquitin-like modifier protein (SUMO) conjugated to one K2P1 subunit to be sufficient to silence the dimeric channels (Plant et al., 2010, PNAS 107). Here, we assess K2P1 sumoylation in cultured rat cerebellar granule neurons (CGN). K2P channels have been posited to produce IKso (the standing outward potassium current responsive to changes in pH and volatile anesthetics) in CGN. First, we developed a fluorescent method to count single mRNA transcripts and showed that those for KCNK1, KCNK3 and KCNK9 (encoding K2P1, K2P3 and K2P9, respectively) were present together in individual CGNs at a ratio of 2:8:1. Next, using antibody-mediated FRET, we observed native K2P1, K2P3 and K2P9 to interact with SUMO1 at the neuronal surface whereas native K2P2 (TREK1) did not. Unlike channels with K2P1, the function of channels with K2P3, K2P9 or K2P2 were found to be insensitive to SUMO1. We reconciled the apparently discrepant findings by showing that K2P1 subunits are incorporated into mixed assemblies with K2P3 or K2P9 (but not K2P2) to form novel two P domain, acid-sensitive (TASK) channels. Channels with just one K2P1 subunit were held in silent reserve at the CGN surface by sumoylation. Accordingly, intracellular application of the desumoylating enzyme SENP1 to CGN doubled the magnitude of IKso (decreasing excitability via expected shifts in resting membrane potential and resistance) and tripled the response to halothane. Because K2P1, K2P3 and K2P9 are expressed together throughout the body and the SUMO pathway is ubiquitous this mechanism of regulation is expected be common.

2095-Plat

Activity-Dependent Transcriptional Regulation of M-Type K^+ Channels by AKAP79/150-Mediated NFAT Actions

Jie Zhang, Mark Shapiro.
UTHSCSA, San Antonio, TX, USA.

M-type K^+ channels, encoded by the KCNQ2-5 family of genes, play key roles in the regulation of neuronal excitability; however, less is known about the mechanisms controlling their expression. Here we propose that neuronal stimulation induces elevated M-channel expression by activation of NFAT transcription factors in response to Ca^{2+}/calcineurin (CaN)-mediated dephosphorylation, orchestrated by A-kinase-anchoring protein (AKAP) 79/150. We observed augmented mRNA for KCNQ2-3 and currents after stimulation of rat sympathetic neurons by either high-K^+ or acetylcholine (ACh), by qRT-PCR and perforated-patch whole-cell clamp. Stimulation also elicited an increase in intracellular [Ca^{2+}] and nuclear translocation of both endogenous and transfected GFP tagged-NFATc1-c2 from the cytoplasm to the nucelus. As evidence that this regulation of M channel expression is NFAT-mediated, exogenous expression of constitutively-active NFAT in sympathetic neurons increased tonic M-channel expression, which was not further increased by high-K^+ stimulation. Moreover, this augmented transcription was suppressed by incubation of the neurons with the CaN inhibitor, cyclosporine A, and the membrane-permeable VIVIT inhibitory peptide, which competes with CaN for binding to NFATs. The involvement of AKAP79/150 in CaN/NFAT regulation of M-channel expression was also studied in sympathetic neurons isolated from AKAP150+/+ and AKAP150-/- mice. We found AKAP150-/- neurons to lack NFATc1 nuclear translocation and augmented expression of M channels after high-K^+ stimulation, which was ''rescued'' by transfection of WT AKAP79 or a non-PKC binding AKAP79 mutant, but not AKAP79 mutants unable to bind CaN. Removal of external Ca^{2+} or addition of nifedipine during stimulation eliminated NFATc1 nuclear translocation and augmented M current, suggesting the role of L-type channels as the activity sensor. Thus, neuronal-activity regulates M-channel transcription, which in turn controls neuronal excitability. This activity-dependent transcriptional regulation of M channels involves a Ca^{2+}-dependent NFAT signaling pathway, which requires AKAP79/150 targeting of CaN.

2096-Plat

Calcium Binding Proteins (CaBPs) Selectively Modulate Ca_V1 versus Ca_V2 Channels

Philemon S. Yang, Manu B. Johny, David T. Yue.
Johns Hopkins School of Medicine, Baltimore, MD, USA.

Calcium-binding proteins (CaBPs) comprise a subfamily of calmodulin (CaM)-like neuronal Ca^{2+}-binding proteins that are widely expressed throughout the brain. Intriguingly, CaBPs have been reported to blunt the CaM-mediated Ca^{2+} feedback regulation in multiple subtypes of Ca_V1, and even Ca_V2 channels (*Nat Neurosci* **5**:210). This modulation of Ca^{2+} feedback potentially influences numerous neurobiological functions, thus motivating ongoing investigation of the mechanism of CaBP effects. Here, in the course of discerning the structural determinants of CaBP operation, we uncovered striking selectivity in the actions of CaBPs for Ca_V1, but not Ca_V2 channels. While mouse mCaBP4 eliminates Ca^{2+}/CaM-mediated inactivation (CDI) of Ca_V1 channels (confirming reports of multiple groups), we now find no such effect on both Ca^{2+}/CaM-dependent facilitation (CDF) of $Ca_V2.1$ channels and CDI of $Ca_V2.1$ and $Ca_V2.3$ channels. Indeed, further analysis revealed the structural basis for such selectivity. Systematic FRET 2-hybrid assays revealed a bevy of CaBP4 binding sites on various intracellular regions of $Ca_V1.3$. As such, we made chimeric channels using $Ca_V2.3$ as the backbone, and substituting intracellular loops from $Ca_V1.3$ to identify functionally relevant mCaBP4 sites. Transfer of the $Ca_V1.3$ carboxyl terminus was sufficient to confer mCaBP4 modulation on $Ca_V2.3$ channels, but CDI was only partially blunted by mCaBP4. By contrast, substitution of the amino-terminus, III-IV loop, and carboxyl-terminus of $Ca_V1.3$ into $Ca_V2.3$ resulted in complete elimination of CDI by mCaBP4. These results suggest that mCaBP4 acts in concert through multiple sites to fully eliminate CDI. Because mouse mCaBP4 induces the most potent effects, we emphasized characterization with this particular CaBP, though other CaBPs may reproduce the same trends. The remarkable ability of CaBPs to selectively modulate only Ca_V1 but not Ca_V2 channels opens new avenues for customization of neurobiological effects, and for development of selective pharmacology targeting Ca^{2+} regulation.

Platform: Membrane Structure II

2097-Plat

Resolving the Structure of Ordered Domains in Single Hydrated Lipid Bilayers by New X-Ray Diffraction Methodology

Roy Ziblat[1], Leslie Leiserowitz[2], Lia Addadi[2].
[1]Harvard University, Cambridge, MA, USA, [2]Weizmann Institute of Science, Rehovot, Israel.

Saturated lipids spontaneously form rigid domain in lipid membranes. X-ray scattering from these domains indicate that their sizes vary between 2-60nm